ORIGINAL ARTICLE

Hemoglobin E Disease and Trait: A Clinico-Hematological Study in a Tertiary Care Centre

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ABSTRACT

Introduction: Hemoglobin E (HbE) is the second most prevalent structural abnormality in hemoglobin (Hb), following sickle cell hemoglobin (HbS). Our aim is to assess the clinical and hematological characteristics HbE disease and trait, incorporating observations from High-performance liquid chromatography (HPLC) and iron profiles. **Materials and Methods:** The study was conducted in the department of hematology, from June 2022 to December 2023. Out of 264 individuals sampled for HPLC, based on their peripheral smear (PS) and complete blood count (CBC) findings, 36 individuals found to have HbE disease. This study involved a comprehensive analysis of the hematological parameters, HPLC findings, and iron profiles of individuals with HbE. **Results:** In our study, we found 14 cases with HbE trait and 22 cases with HbE disease, out of the 264 cases analyzed in HPLC. It included pregnant females who came for routine check-up as well as referred patients suspected to have hemoglobinopathies who showed a range of symptoms, along with normal or slightly increased red blood cell (RBC) count with decreased mean corpuscular volume (MCV), and presence of microcytic RBCs and target cells in PS. Their iron levels were within normal limits, and HPLC revealed elevated levels of HbA2. **Conclusion:** HbE disorders constitute a diverse range of diseases, experiencing a significant global rise especially in people of Assam. Normal or increased RBC count with low MCV on CBC, target cells and microcytosis on PS with a normal iron profile should raise a suspicion of HbE and further investigations like HPLC should be performed.

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INTRODUCTION

Haemoglobinopathies arise due to mutations in the non-coding, coding, or regulatory regions of the globin genes. Structural changes induced by mutations in the globin genes lead to abnormal Hb. These abnormal Hb can be linked to severe disorder or can be clinically asymptomatic [1]. HbE, an autosomal recessive disorder stands as the predominant Hb variant in both Southeast Asia and Northeast India. At position 26 of the beta globin chain, a single-point mutation that substitutes lysine for glutamic acid characterizes this aberrant Hb [2]. HbE disorders can manifest in heterozygous (AE), homozygous (EE), and compound heterozygous states (such as HbE with other abnormal hemoglobins or thalassemias), resulting in clinically diverse phenotypes [3]. The high prevalence of Hb AE red cells worldwide is likely due to their resistance to invasion by Plasmodium falciparum. This mutation also triggers a hidden mRNA splice site, reducing the synthesis of the β-E chain and resulting in a thalassemia phenotype [4]. CBC, HPLC, and deoxyribonucleic acid (DNA) analyses represent diverse diagnostic approaches employed globally to evaluate the prevalence of thalassemia and other hemoglobinopathies [5].

HPLC is a rapid and automated technique known

for effectively identifying a majority of Hb variants and offering accurate measurements of HbA2 and HbF across various Hb genotypes. Nevertheless, the currently approved HPLC methods face limitations in distinguishing HbE from HbA2 [6]. Recognition of this comparatively uncommon Hb variant in this region of India can contribute to improved clinical diagnosis and management of affected individuals. Additionally, it may facilitate advancements in prenatal diagnosis and genetic counseling for these patients. The aim of our study is to assess the clinical and hematological characteristics of individuals with HbE disease and trait, incorporating observations from HPLC and iron profiles. The clinical, hematological and laboratory features of this disorder are also discussed

MATERIALS AND METHODS

This retrospective, cross-sectional study was carried out in the Department of Hematology, in our institute after getting ethical committee approval with reference number (163/04/2024/PG/SRB/SMCH). A total of 36 cases of Haemoglobinopathy E (22 cases of Hb E disease and 14 cases of Hb E trait) were identified among 264 HPLC reports sampled during the period from June 2022 to December 2023. These patients were suspected of having haemoglobinopathy based on demographic details, CBC and PS findings. A comparative study was performed between levels of HbA2, HbF, HbA, Hb, RBC counts, and RBC indices including MCV, Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Packed cell volume (PCV) and Red cell distribution width (RDW).

Inclusion criteria:
1. Individuals presenting with diverse manifestations of haemoglobinopathy in CBC and PS, such as microcytosis, target cells, and unexplained anaemia were included in the study.
2. Individuals from both sexes of all age groups and demographic backgrounds were incorporated.
3. It also included pregnant women who were suspected to have haemoglobinopathy in their routine antenatal check-up.

Exclusion criteria:
1. Individuals identified with haemoglobinopathy, other than HbE trait and HbE disease as identified by HPLC, were removed from the study.
2. Individuals who underwent blood transfusion within 3 months prior to the HPLC study.

The investigation involved a comprehensive analysis of the hematological parameters, HPLC findings, and iron profiles of the participants. The CBC was performed using a 6-part automated analyzer, operating on electrical impedance and flow cytometry. Additionally, PS findings and iron profiles was collected for all cases. HPLC using Bio-Rad D-10 dual mode machine served as the confirmatory test for identifying hemoglobinopathy in the course of this study. Blood samples were obtained in EDTA tubes for CBC, PS, and HPLC, while clotted vials were used for iron studies.

Each type of hemoglobin has a unique retention time. HPLC operates on the principle of cation exchange, utilizing differences in retention times. HbE, an abnormal hemoglobin, is distinguished by analyzing the percentage of HBA2, as HbE shares the same retention time as HbA2 on HPLC but can be identified by its higher concentration. If the HbA2 peak is greater than 4% but less than 9%, it suggests the individual is a beta-thalassemia carrier, without involvement of HbE. If the HbA2 peak falls between 22% and 40%, it is indicative of HbE trait, while a range of 70% to 90% suggests HbE disease or homozygous.

RESULTS

A total of 264 cases sampled for HPLC suspected to have haemoglobinopathy based on their PS and CBC findings, 14 cases of HbE trait and 22 cases of HbE disease were found. Rest of the individuals found to be normal or having other haemoglobinopathies, who were excluded from our study. Out of the 14 individuals with HbE trait, 4 were males and the remaining 10 were females. Additionally, among the 22 cases of HbE disease, 10 were males and 12 were females. The common age group was between 20-30 years in both HbE trait and disease as seen in Table I. The prevalence of HbE is more common in Assam followed by West Bengal and Uttar Pradesh which is depicted in Figure 1. The majority of females were asymptomatic and identified with the HbE variant during their regular antenatal check up. Some dengue positive individuals, presented with symptoms such as fever and myalgia and diagnosed to have HbE variant. Few individuals presented with generalized weakness, easy fatigability and exertional dyspnoea.

Table I: Age distribution for HbE trait and disease.

Age (years)	HbE Trait	HbE disease
0-10 years	1	0
10-20 years	0	2
20-30 years	9	16
30-40 years	4	4

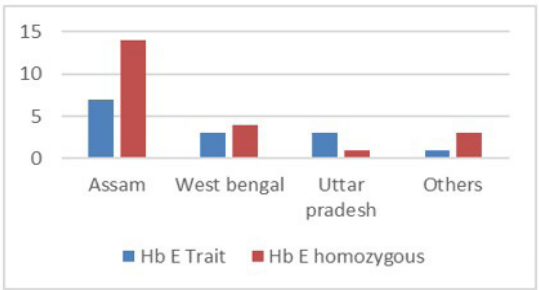

Figure 1: Prevalence of HbE trait and disease in India.

Hematological Parameters:
As shown in Table II, individuals with the HbE trait had an average Hb level of 10.2 ± 3.4 gm/dl, with values ranging from 4.5 to 15.4 gm/dl. The average RBC count was 4.7 ± 1.4 million/mm , and the average hematocrit was 32.2 ± 8.4%. The MCV was decreased in the majority of patients, with an average of 74 ± 10.9 fl, and the MCH was also reduced, averaging 22.9 ± 4.4 pg. For individuals with HbE disease, the average RBC count was 5.2 ± 1.2 million/mm , slightly higher than normal. Their Hb levels ranged from 7 gm/dl to 13.6 gm/dl, with an average of 10 ± 1.9 gm/dl, and the average hematocrit was 30.3 ± 6.9%. The MCV was significantly decreased in all patients with HbE disease, ranging from 54.6 fl to 75.2 fl, with an average of 60.6 ± 3.8 fl. The MCH values were also decreased, ranging from 17.5 pg to 23.7 pg, with an average of 19.3 ± 1.2 pg. The MCHC was normal in most cases of both HbE disease and HbE trait. Additionally, the white blood cell count and platelet count were within the normal range in most individuals with HbE disease and HbE trait.

Table II: Hematological Parameters for HbE trait and disease

Hematological Parameters	HbE trait	HbE disease
Hemoglobin (g/dl)	10.2 ± 3.4	10 ± 1.9
RBC count (million/mm^3)	4.7 ± 1.4	5.2 ± 1.2
Hematocrit (%)	32.2 ± 8.4	30.3 ± 6.9
Mean Corpuscular Volume (fl)	74 ± 10.9	60.6 ± 3.8
Mean Corpuscular Haemoglobin (pg)	22.9 ± 4.4	19.3 ± 1.2
Mean Corpuscular Haemoglobin Concentration (g/dl)	30.8 ± 2.1	31.9 ± 0.7
Red cell Distribution Width	18 ± 5.2	17.2 ± 3.6

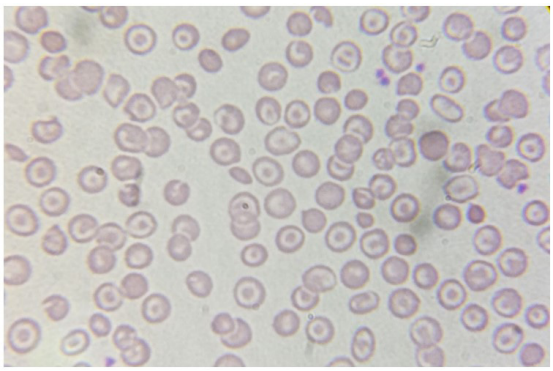

Figure 2: Peripheral blood film showing microcytic hypochromic RBCs, target cells and elliptocyte.

Peripheral blood film examination:
All the individuals with HbE disease showed microcytic hypochromic RBCs with anisopoikilocytosis. In 90% of the cases addition to microcytic hypochromic RBCs, target cells and elliptocytes were also seen as seen in Figure 2. Among the HbE trait individuals, 60% of them showed microcytic hypochromic RBCs with anisopoikilocytosis and only 5% of the cases showed target cells and elliptocytes, and remaining cases showed normocytic normochromic blood picture.

Iron profile:
Most cases had normal serum ferritin levels, and both serum iron and total iron binding capacity (TIBC) were within normal limits. However, a few individuals (4 out of 36) exhibited decreased serum ferritin and iron levels along with increased TIBC.

HPLC findings:
The range of HbA2, HbF and HbA0 were determined by HPLC method as shown in Table III. The value of HbA2 in HbE trait ranged from 23.1%-35.7% with a mean of 32.2 ± 10.3%; while in HbE disease it ranged from 85.4%-98.1% with a mean of 91.3 ± 4.8% on HPLC.

The patterns of HPLC seen in normal individuals, HbE trait and HbE disease cases is shown in the Figure 3. The HbA2 peak is increased in HbE variant cases compared to normal individuals, and also it is much higher in individuals with HbE disease than HbE trait.

Table III: Range of HbA2, HbF and HbA0 determined by HPLC method in HbE trait and disease

Parameters	HbE Trait	HbE Disease
HbA2	32.2 ± 10.3	91.3 ± 4.8
HbF	1.3 ± 0.6	4.6 ± 2.6
HbA0	56.6 ± 20	5.9 ± 0.6

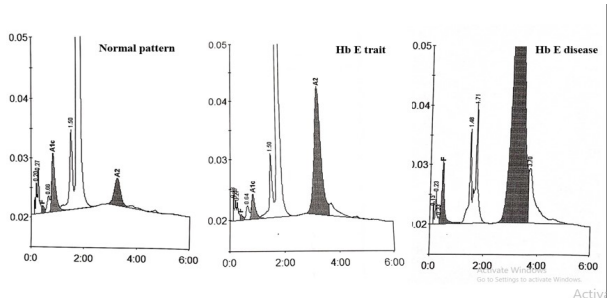

Figure 3: Patterns of HPLC seen in normal individuals, HbE trait and disease.

DISCUSSION

HbE, the fourth abnormal hemoglobin to be discovered, was first identified in the year 1954 [7]. In the specific context of North Eastern India, including Assam, HbE emerges as a noteworthy hemoglobinopathy. Despite originating from a single point mutation, the development of this hemoglobinopathy results in a notable phenotypic heterogeneity due to their nature as autosomal recessive disorders. The reasons for significant phenotypic diversity in HbE disorders remain largely unknown, indicating gaps in understanding the underlying genetic or environmental factors. On one end of the spectrum, both homozygous and heterozygous forms of HbE exhibits minimal symptoms, while the compound heterozygous form presents a clinical picture indistinguishable from thalassemia major. The oxidative stress and elevated temperatures can precipitate unstable

HbE, leading to red cell damage. It is now recognized that the coinheritance of α or β thalassemia with HbE, particularly in the form of compound heterozygosity, results in a clinical syndrome of varying severity, particularly notable in South East Asia due to complex interactions [8].

In the heterozygous state, the presence of HbE results in minimal morphological abnormalities in RBCs and normal red cell indices. Conversely, the homozygous state is characterized by microcytic hypochromic red cells with significant morphological abnormalities, such as target cells, leading to mild anemia in most cases. Additionally, RBCs in the homozygous state show reduced survival, as indicated by diminished osmotic fragility tests and a hemolytic picture in the PS. Patients may also occasionally present with splenomegaly, hepatomegaly, cholelithiasis, and hyperbilirubinemia. A case report by Bhattarai U et al. described a patient with homozygous HbE disease exhibiting pallor, pancytopenia, microcytic anemia, indirect hyperbilirubinemia, target cells, and a hemolytic picture in the PS. An abdominal ultrasound revealed massive splenomegaly with multiple well-defined echogenic lesions throughout the splenic parenchyma, leading to the recommendation for splenectomy and genetic screening [10].

CBC analysis, including assessments of blood counts, Hb levels, RBC indices, as well as HPLC and DNA analysis, are employed globally for diagnosing the predominance of thalassemia and other hemoglobin disorders. HPLC is especially efficient in separating different types of hemoglobins, allowing for precise measurement of HbA2 and HbF levels, and detecting the majority of Hb variants [3]. HbE disorders can be present along with α or β thalassemias, as well as iron deficiency anaemia. While an HPLC study can identify HbE/β thalassemia but not α thalassemias, further analysis through DNA study is necessary in such cases [11]. Similar to other hemoglobinopathies, conducting an iron study is crucial to avoid inaccurately low values of RBC parameters, particularly in rural and antenatal populations. HbE disorders has a paradoxical nature, with a spectrum of behaviours ranging from favorable to severe, and the level of HbE does not consistently correlate with disease severity [8]. Our study demonstrates that RBC indices acquired from automated cell counters and PS findings can offer valuable insights into the diagnosis of hemoglobinopathies, with the specific type being confirmed through HPLC analysis.

The significance of HbE is underscored by certain characteristics:

Individuals with HbE exhibit a microcytic hypochromic profile, which can be mistaken for thalassemia or the early phases of iron deficiency.

Individuals who inherit both HbE and β-thalassemia genes suffer from severe anemia, resembling the symptoms seen in thalassemia major [3].

A study by Sirichotiyakul et al. found that homozygous HbE disease does not significantly increase the risk of common adverse pregnancy outcomes but does significantly raise the risk of fetal growth restriction. HbE, which differs from normal hemoglobin like HbA, is partly linked to abnormal fetal growth. However, its precise effect on the oxygen dissociation curve at the placental beds is not well understood [9]. Despite the significant burden of hemoglobinopathies, India has not established a carrier screening program, although national guidelines for their prevention and control were issued by the Ministry of Health and Family Welfare in 2016. Carrier screening allows for informed reproductive choices and has been an effective strategy in many countries with high rates of hemoglobinopathies. Recent research indicates that the cost of implementing a screening program is less than 4% of the total expenditure required for patient treatment [12].

The limitations of this study included: (1) it is a retrospective study, (2) the study group had a relatively small sample size, (3) the absence of long-term follow-up data on clinical outcomes for patients with HbE disease and trait limits insights into disease progression and management.

CONCLUSION

HbE disorders constitute a diverse range of diseases that are experiencing a significant global rise especially in people of Assam. The underlying reasons for the significant phenotypic diversity largely remain unknown. A normal or increased RBC count with decreased MCV or MCH on a CBC, microcytosis, and target cells on a PBF with a normal iron profile should prompt suspicion of HbE. Further investigations, such as HPLC or capillary zone electrophoresis, should be performed. The current study highlights the significant prevalence of HbE within our tertiary care hospital due to migration of population, underscoring the importance of raising awareness among healthcare providers and the general public.

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